DEVELOPMENT OF FUNGAL PROTEOMIC TECHNIQUE IN FOOD TECHNOLOGY

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Abstrak

Fungi merupakan mikroorganisme yang berperan dalam kehidupan manusia, organisme ini dapat menguntungkan maupun merugikan manusia, hewan, tumbuhan, dan lingkungan. Dalam industri pangan kapang dan khamir diaplikasikan untuk produksi pangan dengan fermentasi seperti tempe, kecap, bir, koji, sake, miso dan produk pangan yang lain. Dengan perkembangan ilmu bioteknologi fungi juga dieksploitasi untuk produksi enzim, anti bakteri, dan anti jamur, serta dipergunakan untuk produksi asam-asam organik.

Dengan melihat peranan *fungi* yang besar dalam kehidupan manusia, penelitian tentang fungi berkembang dengan pesat. Salah satu penelitian yang banyak dilakukan adalah proteomik. Proteomik merupakan suatu penelitian identifikasi, separasi, maupun kuantitatif dari protein yang dihubungkan dengan gen yang bertanggung jawab akan pembentukan protein secara spesifik. Cara pemisahan yang paling banyak dilakukan adalah dengan gel elekroforesis SDS PAGE, untuk kemudian dianalisis dengan spektrometri massa MALDI TOF. Hasil yang didapatkan dari spektrometri ini kemudian diidentifikasi dengan darta-data genome yang telah tersedia. Faktor yang harus diperhatikan adalah ekstraksi protein yang tepat, prosedur elektroforesis yang benar, serta identifikasinya.

Proteomik merupakan teknologi baru yang masih berkembang, teknik ini penting karena dapat mengetahui gen yang berpengaruh terhadap sekresi metabolit sekunder protein yang dapat berguna maupun membahayakan. Sehingga dengan mengetahui faktor yang berpengaruh, maka untuk produk sekresi yang berguna kita dapat meningkatkan produksinya dengan modifikasi gen maupun kondisi optimumnya.

Kata kunci: fungi, proteomik, gen, elektroforesis, spekrometri massa.

INTRODUCTION

Fungi are eukaryotic microorganism that commonly involve in our daily life. They could act both as harmful and beneficial agents in plants, animal, human, and environment (Abbas, 2005). They had long been used in food production and also play a significant role in human therapeutics and chemical industries. In the other hand several species of fungi could act as pathogenic agents (Kuhn et al., 2003) that threats human health via contaminating food and feed stuffs and produced mycotoxin as it secondary metabolites which is highly toxic in human body (King and Prudente in Abbas, 2005)

In food production, fungi have long been applied in the traditional food fermentation industry such as tempeh, soy sauce, koji, beer, rice wine (sake), soybean paste (miso) and other food process (Ng, 2004). With the growth of biotechnology industry, fungi have been exploited as a source of various commercial enzymes, antibacterial and antifungal agents (Adrio and Demain, 2003), and also organic acid (Magnuson and Lasure, 2004). But fungi also act as pathogenic agent, for example genus of *Aspergillus* i.e. *A fumigatus* and *A flavus* could contaminate corn, peanuts, flour, and other food (Carlile et al., 2001). Another genus is *Fusarium* which could contaminate citrus, tomato, banana, and other fruit crop (Logrieco et al., 2002). All the infected products will result in chronic toxicity and cancer if consumed by animals or human. In developing countries, fungal infections have become a problem because of limited knowledge in pre harvest stage and also insufficient post harvest handling, storage, and distribution (Shier et al. in Abbas, 2005).

According to both of their special role, which involved billion of dollars for food industry, industrial biotechnology and disease prevention, fungi have been widely investigated. In recent years research in the area of toxigenic filamentous fungi and fungal biotechnology increased rapidly (Kim et al., 2007). Many research conducted to sequence the genomes of fungi in order to find the gene that responsible of secretion of secondary metabolites which could be harmful or beneficial of fungi (Nevalainen et al., 2005., Robson et al., 2005., Keller et al., 2005).

Nowadays research in fungi developed to post genomics area. Interest directed towards the proteins that the genomes encode (the proteome). The short definition of proteome analysis is identification, separation, and quantitation of proteins (Wasinger et al, 1995) and Wilkins (1995) define the concept of proteome analysis as the analysis of the entire PROTEin complement expressed by a genOME, or by a cell or tissue type. Proteome also defined as the complete set of proteins that is expressed, and modified following expression by the genome at given time point and under given conditions in a cell. For example Perlinska-Lenart et al (2005) analysed protein production and secretion in an *Aspergillus nidulans* mutant impaired in glycosylation compared with its native.

Proteomics become the popular approach for scientist because it allows investigation on a specific location of cell which responsible for phenomena occurred in molecular based of fungi (Acero, et al., 2007). Researchers to date have used several techniques to study fungal proteomic in order to get a better view of sequenced proteins and to identify its function. However research in fungal proteomics faced several problems for example difficulties in obtaining fungal protein and lack of database protein sequenced (Carberry and Doyle, 2007).

This review documents the research efforts in proteomic analysis of various species of fungi.

Fungal Proteomic Technique

Emerging fungal proteomics in recent years it increases in number of research and reviews. It attracts researcher's attention because proteomic has proven to be the most powerful method for identification of proteins in complex mixtures and is suitable for the study of alteration of protein expression in an organism under varying environmental conditions (Medina et al., 2005).

Proteomic consist of two main analyses which are protein separation and identification. In earlier studies researchers used Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) which separated protein according to their molecular weights (O'Farrell, 1975), but this technique had several limitations such as the presence of several different proteins in a single stained band which cause misinterpreting of protein. (Graves et al, 2002., Figeys, 2005). The most common method used by researcher on fungal proteomic is two dimensional gel electrophoresis (2DE) which is gel-based separation of proteins by the orthogonal properties of isoelectric focusing (IEF) and molecular weight for protein separation, followed by Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) mass spectrometry (MS) one of the most common types of mass spectrometry devices used to perform peptide mass fingerprinting (PMF) (Pitarch et al, 2002., Kniemeyer et al, 2005., Carberry et al, 2006., Oda et al, 2006., Fernandez-Acero et al, 2006., and Schmidt et al, 2008). Either whole or fragmented proteins are ionized by a laser in the presence of crystallized matrix and are subsequently subjected to an electrical field to measure the amount of time that it takes for the particles to travel a known distance to correlate the time-of-flight of a particle with its mass to charge ratio (Giorgianni, et al., 2002). Medina and Francisco (2008) proposed LC-MS/MS technique to identify secreted protein from *A. flavus* and conclude that this technique is more effective if the genome sequence is less known.

Fungal proteomic analysis consist of several steps that researcher has to focused on Figure 1.

Design of Experimental Culture Condition

Typically this is the first step conducted in fungal proteomic research. Several research were held to compare different culture condition on fungal proteomic. In this step, to minimize errors of the experiment, all culture should be made similar except the targeting culture condition (Carberry and Doyle, 2007), for example carbon source, pH, etc (Zilm, et al., 2003). Kniemeyer et al (2005) proposed glucose and ethanol as the carbon source of *A. fumigatus*, while rutin and non rutin culture conditions of *A. flavus* were conducted by Medina et al (2006). *A. Oryzae* proteomic research conducted by Oda et al. (2006) compared protein analysis from solid and sub merged culture conditions, and Sato et al. (2007) compared liquid and solid culture conditions. Hughes et al. (2007) compared *Mycobacterium avium* grown in vitro and isolate from clinical cases. Condition exposed to certain physiological states (Wang et al, 2005., Apraiz et al, 2006., Behr et al, 2007).

Fungal Proteomic Extracts

In fungal intracellular and sub cellular proteomic, cell wall lysis is the most important factor to yield a sufficient intracellular or organelle protein, because filamentous fungi had been known to have a strong cell wall (Maheshwari, 2005). This cell wall could be a problem in IEF because it has an ability to bind several proteins via electrostatic interaction and its polysaccharide components could block the pores of immobilized pH gradient (IPG) gel which resulted in horizontal streaking on 2DE gels (Duran, et al., Herbert, 2006). Therefore this step is very important to 2DE process. Several technique were used for cell lysis, for example mechanical lysis using glass beads (Pitarch et al, 2002., Nandakumar et al, 2003., Schmidt et al, 2008), enzymatic lysis with lyzozyme (Dhingra, 2005) and chemical lysis (Asif et al, 2006). This point of stage is crucial and difficult, therefore protoplast generation of *Tyromyces palustris* were conducted by Shimizu and Wariishi (2005) to avoid the difficulties of lysing the cell wall. Carberry et al (2006) were combined lysis buffer treatment containing Tris-HCl, NaCl, Ethylene diamine tetraacetic acid (EDTA), glycerol, dithiothreitol (DTT), phenylmethylsulphonyl fluoride (PMSF), and pepstatin A and accomplished by grinding in liquid nitrogen. However the most common methods used for lysing cell wall were grinding in liquid nitrogen using mortar and pestle (Kniemeyer, 2005) or in potter homogenizer.

The next step is precipitation of protein. Tricholoracetic acid (TCA) is the most common chemical used for precipitation. This stage is important because it remove undesirable compound which could disturb isoelectric focusing process (Jeffries, 2000). After cell lysis, precipitation, centrifugation and removal of undesirable compound, the sample needs to be resolubilized, however TCA treatment in earlier step makes solubilizes precipitate hardly to obtain. Several researcher were tried to improve the solubilization at later stage, using phosphate buffer (Nandakumar, et al., 2003). Acidic solvent extraction was used by Herbert et al. (2006) in pre-precipitation process, and Kniemeyer et al. (2006) used zwitterionic detergents and sodium hydroxide after precipitation stage to improve the solubility of sample (Kniemeyer et al, 2006).

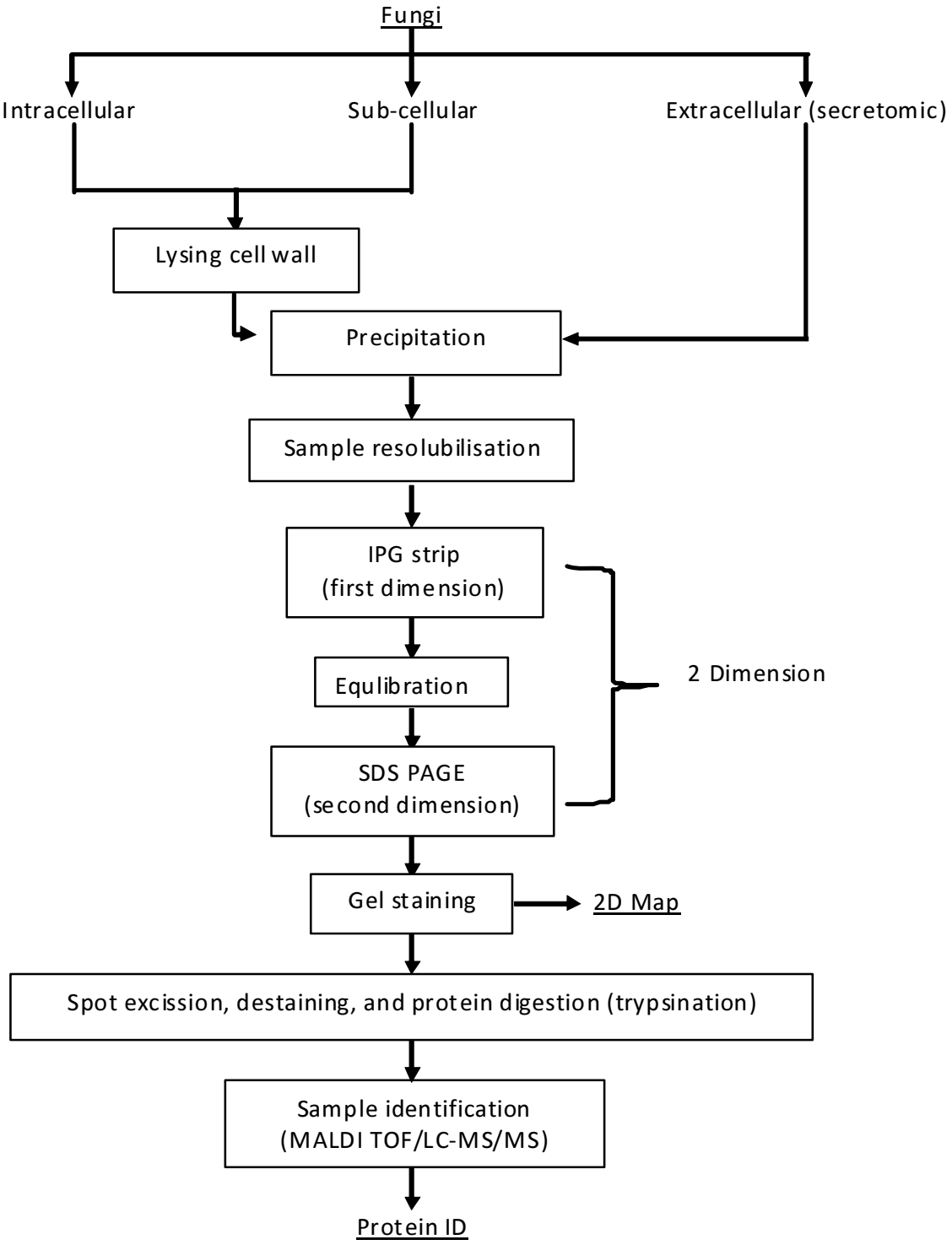

Figure 1. Fungal Proteomic Analysis

The resolubilization of sample requires uses of solubilisation/denaturation (SD) buffer for separation of individual protein. This buffer consists of chaotrophe, reductant, detergent, ampholytes, and protease inhibitor (Jefferies et al., 2000). Research reported that urea and thiourea are the most common chaotrophes used in fungal proteomic. Their action is through the disruption of hydrogen and hydrophobic bonds. Another constituent of buffer is a reductant, it acts through breaking any disulphide bridges that exist within a particular protein. DTT is the most common reductant and used by almost all researcher. Detergent was used to disrupt membranes, to solubilize lipids and delipidate proteins bound to vesicles or membranes. The detergents must have strong character to solubilize the target compound. The most widely use detergent for fungal protein buffer were 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and Triton X-100. Ampholytes were used to improve protein solubilisation; in ultracentrifugation process ampholytes help to precipitate nucleic acids, and they can also act as ion scavenger, which scavenge cyanate ions. The ampholyte mixture used should reflect the pH of the IEF gel to be run. All constituents required should include in the buffer system, but their proportions were different according to the species of fungi analysed because every species of fungi have a specific characteristic of cell and protein constituents. For example a mixtures of urea, CHAPS detergent, DTT, and Bio-Lyte carrier ampholytes were used by Medina et al. (2005) to solubilize sample protein secreted by Aspergillus flavus. Another chemical used are Thiourea and Triton X-100. Before applied to 2-DE, protein content of the sample should be analyzed. The conventional methods of protein analysis (Bradford) were commonly used by researcher.

2 Dimensional Gel Electrophoresis (2DE)

2 DE has been widely applied to find proteins that are differentially expressed between sample and groups of samples. This 2 DE is recently the most common technique used in proteomics (Gorg, et al, 2004). It is widely recognized for its compatibility with mass spectrometry to identify protein fractions of a cell or specific organelles, including fungal proteins. In the first dimension, proteins were separated by its isoelectric points. Recently commercially available immobilized pH gradient (IPG) strips are widely used by researcher because they are more stable as the gel is plastic backed and the pH gradient is fixed. This leads to a mechanically stronger strips and stability of pH gradient (can't drift). This IPG is commercially available (Bio-rad, Amersham, and others) in various ranges of pH's (examples: pH 3-10, 4-7, 3-6, 5-8, 7-10) and different lengths (7, 11 and 17cm). When suitable IPG strip is found, firstly, it is reswelled using a mixture of protein extracts with a loading buffer stated previously (Sanchez, et al, 1999). The proteins will migrate to their isoelectric point when an electric field is applied. As proteins migrate to their respective isoelectric points, they pick up or lose protons. As the proteins continue to migrate, the net charge on the proteins and their mobility decreases and eventually they come to a point where their net charge is zero and they stop moving in their isoelectric points.

Prior application of IPG strip to the second dimension which is SDS PAGE, the IPG strip must be equilibrated in a buffer containing SDS (sodium dodecyl sulfate), which is a detergent that will bind to the protein in a ratio of one SDS molecule for every two amino acids. This binding capability causes the protein to once again become negatively charged in proportion to its molecular weight, which is essential for migration in the second dimension. The proteins are also reduced and alkylated using DTT and iodoacetamide (IAA).

DTT reduces the disulfide bonds (R-S-S-R) found in proteins. The resulting –SH groups are then alkylated with iodoacetamide, which attaches an alkyl group to the –SH ($R\text{-}S\text{-}CH_2\text{-}CONH_2$) to prevent reformation of the disulfide bonds. After equilibration process, the IPG strip is then positioned on the upper edge of SDS PAGE gel. The second dimension acts like a molecular sieve so that the small molecules can pass more quickly than the large ones. An electric field is then applied across the gel and the proteins migrate into the second dimension where they are separated according to their molecular weights.

After the two process of electrophoresis is complete, the resulted gels were stained and scanned. Commasie Brilliant Blue (Patton, 2002) was commonly used by researcher for gel staining. Then encircled spots were excised manually and washed with solution to completely remove the commasie (destaining). The excised spots then digested by enzyme to yields peptides. Trypsin was enzyme that commonly used to digest the gels. The resulted peptides are then applied to mass spectrometry or liquid chromatography tandem mass spectrometry for identification. Mass spectrometry popularly used in protein identification because they provide structural information of protein in the forms of peptides and amino acid sequences (Graham et al, 2007). The common methods for protein identification are MALDI-TOF, Electrospray Ionization (ESI)-MS, and LC-MS.

Protein Identification

Protein and peptides analysis with MS could result in peptide mass fingerprinting (PMF) and fragmentation spectra. PMF is popularly used by researcher because the PMF compares unknown peptides from sample with database of theoretical predicted masses. However this is also the handicap of MS method; that is if there is lack of sequenced protein database of a species we could not identify the protein spots. Researcher in the area of fungal proteomic also faced with this condition because of lack of information in the fungal protein sequence database.

Because of the importance of fungi in human life, in recent years several articles on the topics are published and research on genome sequencing of fungi increases. This condition supports successful identification of fungal proteins. We can find a database of sequenced fungal genomes in http://www.broad.mit.edu, COGEME http://cogeme.ex.ac.uk, or URGI http://urgi.versailles.inra.fr.

Identification of proteins conducted by Medina et al. (2006) using SEQUEST program that allows correlation analysis of experimental data with theoretical spectra generated from known protein sequences. Medina et al were searched database from NCBI non redundant protein, Swiss-Prot databases, The Wellcome Trust Sanger Institute (www.sanger.ac.uk), and http://www.tigr.org. The functions of annotated proteins were assigned by BLAST homology searching. We also could find *A. fumigatus* sequenced genome at http://www.cadre.man.ac.uk.

The National Institute of Advanced Industrial Science and Technology (http://oryzae.cbrc.jp/ and www.bio.nite.go.jp/dogan/Top) provide sequenced genome database from *A.oryzae*. Identification of PMF and peptide fragmentation spectra also can be done with MASCOT interface (MASCOT 2.1.0, Matrix Science, London, UK) or ProFound (Genomic Solution, USA)

Statistical Analysis

Statistical analysis in 2 DE is important according to reproducibility of analysis. We have to determine the number of replications we will use for our experiments. A small number of replications might not describe our protein results, but too many replications also make greater variability of the results. Research conducted by Asirvatham et al. (2002) used ten replicates for their analyses, in other work Hubalek (2004) performed two replicates every strain. Three repli-

cates were the most commonly used by researchers (Hernandez, Jorge, Ornstein, Donnely in Acero, 2007).

Intracellular, Sub-cellular, and Extracellular Proteomic

Figure 1 shows that fungal proteomics research divided to three main subjects which are intracellular, sub-cellular, and extracellular proteomics. Intracellular study relate to whole cell cytoplasmic proteomic, sub-cellular study identify protein in cell envelopes and specific organelles i.e. mitochondrial protein, and extracellular proteomic relate to protein secreted by fungi.

Intracellular

A. fumigatus intracellular research were conducted by Kniemeyer et al. (2005), who focused on optimization of a 2-D gel electrophoresis protocol. The result were then identified with MALDI TOF-MS. 37 spot were identified by MALDI TOF, The identified proteins involved in glycolysis, TCA cycle, glyoxylate cycle, ethanol utilization, gluconeogenesis, and amino acid metabolism.

Carberry et al. (2006) also analyzed intracellular proteins from *A fumigatus* with MALDI mass spectrometry. A total of 180 spots excised from 2D-PAGE gels. From these result, 50 distinct proteins were identified by MALDI MS. The majority of identified protein involved in energy production. It is claimed that the result represent one of the ûrst detailed identiûcation of the most abundant intracellular proteins of *A.fumigatus*.

Acero et al. (2006) provided intracellular proteomic research from *Botrytis cinerea* using 2 D electrophoresis and MALDI-TOF and ESI IT MS/MS. They detected around 400 spots in 2 DE. Twenty-two protein spots were identified by MALDI-TOF or ESI-IT MS/MS. Some of them corresponded to forms of malate dehydrogenase (MDH) and glyceraldehyde-3-phosphate dehydrogenase (GADPH). Two more spots matched a cyclophilin and a protein with an unknown function. MDH, GADPH, and cyclophilin have been related to the virulence factor of *B.cinerea*. This publication claimed to be the first proteomic analysis of *B cinerea.*

Roger et al. (2006) provided different expressions of intracellular proteins from azole resistance *Candida glabrata* compared to its native. They used 1DE and 2DE for protein separations and identified the proteins with MALDI TOF/TOF. They detected Erg11p in azole resistance *C glabrata*. This critical enzyme in the ergosterol biosynthesis pathway represents the major target of the azole antifungal agents. Homologues of several proteins which known involved in response to oxidative stress also up regulated in azole resistant strain.

Research on *C glabrata* protein characterization towards responses to change in ambient pH were conducted by Schmidt et al. (2008). *C glabrata* were cultured in pH 4; 7,4; and 8. They used 2 DE and MALDI TOF for protein identification. They identified 272 protein spots and 172 spots were identified, indicating that the proteins had significant changes in expression levels. In alkaline conditions (pH 7,4 and 8), the higher levels of proteins involved in stress responses and protein catabolisms were found, whereas proteins which play roles in glucose metabolism, TCA cycle, respiration, and protein synthesis were express in lower levels. This report proposed that *C.glabrata* perceives low pH as less stressful than high pH.

Study in *F graminearum, Ustilago maydis, Phytophthora sojae,* and *Rhizoctonia solani* proteins were performed by Padliya et al. (2006). The reports goals were to determine whether LC-MS/MS could be used to identify peptides and proteins from complex plant pathogens as part of a screening process. And it stated that LC-MS/MS were successfully detected proteins from targeted fungi.

Qin et al. (2006) provided an antioxidant protein and a hydrolytic enzyme identified from *Penicillium expansum* under oxidative stress with

2DE and ESI-Q-TOF MS/MS. Borate containing media were used to identify the stress responses. Several proteins related to stress responses (glutathione S-transferase, catalase, and heat shock protein 60) and basic metabolisms (glyceraldehyde-3-phosphate dehydrogenase, dihydroxy acid dehydratase, and arginase) were identified in the cellular proteome. Two antioxidant enzymes (glutathione S-transferase and catalase) exposed a reduced level in borate containing medium, The results proposed because borate treatment increased levels of reactive oxygen species (ROS) and oxidative protein carbonylation (damaged proteins), which are a precursors of oxidative stress. Therefore, reduced levels of antioxidant enzymes indicate that catalase and glutathione S-transferase are important in scavenging ROS and protecting cellular proteins from oxidative damages.

Subcellular (Sub-proteome)

I used term subcellular proteomic to describe proteomic research in a part of fungal cell or its specific organelles. Hernandez-macedo et al. (2002) provided proteomic analysis of plasma membrane and outer membrane of *P.chrysosporium* and *L edodes* and detected protein spots with 1D SDS PAGE.

Cell wall proteins from *Candida albicans* were reported by Pitarch et al. (2002). 2 DE were used to separate protein and MALDI TOF/MS for their identifications. Cell wall of *C albicans* were isolated and applied to 2DE. The results show that a large number of proteins were involved in cell wall construction, and this research were claimed to be the first report that provide 2DE reference maps of *C albicans* using immobilized pH gradient. Identification and characterization of cell wall proteins of *Saccharomyces cerevisae* were conducted by Yin et al. (2005).

Mitochondria proteome analysis conducted by Grinyer et al. (2004). The group successfully provided protocols for sample preparation, fractionation, and protein maps of mitochondrial proteome from *T.harzianum*. Twenty five proteins were identified from mitochondria and were known to play a significant role in TCA cycle, chaperones, protein-binding and transport proteins.

Schmitt et al. (2006) also provided protein data from mitochondrial outer membranes of *N. crassa*. They used 1D SDS PAGE and MALDI TOF and LC-MS/MS to successfully identified 30 proteins, which some of them involved in transportation and mitochondrial morphology.

Although proteomic analysis from subcellular or organelles were difficult, there is always a possibility to conduct experiments with a good result if protocols for separation and fractionation of organelles are ready to use. The key steps for proteomic analysis of specific organelles are sample preparation and fractionation. This subcellular proteomic is important because we can identify specific protein that responsible in molecular action of the specific organelles.

Extracellular (Secretome)

In this review, we restrict the term extracellular protein as a protein secreted by fungi. Protein secretion in fungi plays an important role, especially in nutrition. Fungi are able to secrete various structural proteins and enzymes which are industrial biotechnology sources as commercial enzyme and drugs (Iwashita, 2002., Breakspear et al. 2007).

Zhu et al. (2004) analyzed secreted protein from *A oryzae* during conidial germination. SDS PAGE and peptide mass fingerprinting method were used to identify protein spot. Taka-amylase A (TAA), glucoamylase (GLAA), and aspergillopepsin A (PEPA) were identified by peptide mass fingerprinting as the main protein secreted by A oryzae during conidial germination.

Medina et al (2006) were analyzed secreted protein from *A flavus* with nano LC-MS/MS growth in different media. They found 50 distinct spots from rutin media, 19 spots from glucose media, and 14 spots from potato dextrose media. Data shows that protein detected from total of

three different media, 27% were unknown or hypothetical protein, 27% were protein that play role in carbohydrate metabolism, 22% proteolysis and peptidolysis protein. 19 unique proteins found in rutin media, 10 and 2 unique protein found in glucose and potato dextrose media, respectively.

Proteomic analysis of extracellular proteins were also conducted by Oda et al. (2006) on *A oryzae* grown under submerged and solid-state culture conditions. They used two-dimensional electrophoresis, and protein spots were identified by peptide mass fingerprinting using MALDI TOF. They analyzed 85 spots from the solid-state cultures and 110 spots from the submerged cultures. 29 proteins were identified. From the conclusion, we know that culture conditions may play a significant role in fungal proteins production.

Extracellular proteomic research of *Fusarium graminearum* were reported by Paper et al. (2007). They were focused on in vitro study from 13 different culture media and *in planta* which used wheat plant. 1D SDS PAGE and MS/MS were used for protein separation and identification. A total of 289 proteins (229 in vitro and 120 in planta) were identified. In planta analysis produced 49 unique proteins which unidentified in in vitro condition. 91-100% protein from in vitro had predicted signal peptides, but in *in planta* condition only 56%. Thirteen non secreted proteins found only in *in planta* were single-copy housekeeping enzymes including enolase, triose phosphate isomerase, phosphoglucomutase, calmodulin, aconitase, and malate dehydrogenase. For this unique protein from *in planta,* it is concluded that significant fungal lysis occurs during pathogenesis. Several of the proteins secreted by animal pathogenic fungi *in planta* have been reported to be potent immunogens, and therefore could be important in the interaction between *F.graminearum* and its host plants.

Qin et al. (2007) also provided extracellular protein from *Penicillium expansum*, and the authors found repression level of polyglacturonase in borate containing medium. This indicated that polygalacturonase also related to oxidative stress response.

Future of fungal proteomic in food safety and technology

The increase of attention in food safety according to human health had led researcher to develop a technology that can be used in preventing food contaminants that could be harmful to human body (Wargo and Hogan, 2007). One of the harmful contaminants is fungi. Fungi can secrete a secondary metabolite called mycotoxin which is cause several disease in human (e.g. cancer, nephro- and hepatotoxicoses). Fungi could attack our daily foods stuff, for example cereals and fruits. This secreted metabolites also resistant to heat process, therefore it could present in the final product like our cereal breakfast, snack, etc. Mycotoxins were also found in milk from cattle that feed with plant or plant products which have been attacked by fungi. According to their threatening effects, authorities proposed a regulation in mycotoxin containing products, but the best way to reduce disease risk by mycotoxin is to control or even prevent food and feed stuff from contaminants.

In this step proteomic will play an important role years to come, because as we knew that in the early contaminating process, both of fungi and its host (for example cereal) will produce several proteins and enzymes (proteome) including anti fungal and anti cereals proteins. Responding to fungal attack, cereals produce proteins and enzymes which could degrade fungal mycelium and this would affect the production of fungal secondary metabolites and extracellular enzyme which are needed by fungi to grow and resist in cereals. Proteomic analysis will enable us to identify proteins and enzymes as an individual compound and to reveal its function. Therefore, a study of interaction between fungi and their hosts in molecular base can be done, and as a final purpose, myc-

otoxin production can be controllable or even preventable through inhibiting of fungal germination which can be done by suppressing fungal germination factor and increasing antifungal factors from cereal or another host.

Proteomic also will have a significant role in food technology especially fermentation based food. As known for hundreds years that fungal have been applied in food to produce several food products, recent years several kinds of fungi reputed as GRAS (generally recognized as safe) and commercially exploited all over the world. Proteomic analysis will enable us to identify enzymes and proteins that play a major role in secretion of metabolites for food productions. Therefore, modification in several aspects (pH, temperature, culture condition, etc) in order to increase these useful chemicals secretion in deeper and specific view will be enabled.

Concluding remarks

Fungal proteomic studies are increasing and it might continue for the next decades. Basically sample preparation is the main problem in this study. Therefore, proper sample preparation, improvement of mass spectrometry technology, and the availability database of fungal protein were the keys of success in identifying fungal proteins. Intensive research on fungal proteomics leads to research on fugal toxigenic secondary metabolites suppression can emphasize factors responsible for the actions, and their applications in food technology.

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